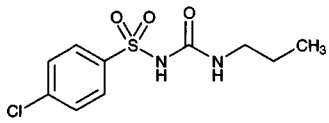
Chlorpropamide



Molecular formula: $C_{10}H_{13}ClN_2O_3S$
Molecular weight: 276.74
CAS Registry No.: 94-20-2
Merck Index: 2239
Lednicer No.: 1 137

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 ng tolbutamide + 500 µL 1 M HCl + 8 mL chloroform, shake on a reciprocal shaker, shake for 10 min in a reciprocal shaker, centrifuge at 2000 g for 15 min. Remove 7 mL of the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 µL 3 mg/mL dinitrofluorobenzene in n-butyl acetate, heat at 120° for 30 min, evaporate to dryness under a stream of nitrogen at 60°, dissolve the residue in 100 µL mobile phase, inject a 30-70 µL aliquot. (Recrystallize dinitrofluorobenzene from diethyl ether. Prepare solutions weekly, store at 4° in the dark.)

HPLC VARIABLES

Column: 125 × 4.6 5 µm C8 (Perkin-Elmer)
Mobile phase: MeCN:water 50:50 containing 0.15% phosphoric acid
Flow rate: 1.5
Injection volume: 30-70
Detector: UV 350

CHROMATOGRAM

Retention time: 6.2
Internal standard: tolbutamide (4.5)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: glyburide
Noninterfering: acetaminophen, aspirin, diazepam, chlordiazepoxide, quinidine, phenytoin, theophylline, phenobarbital

KEY WORDS

plasma; derivatization

REFERENCE

Zecca,L.; Trivulzio,S.; Pinelli,A.; Colombo,R.; Tofanetti,O. Determination of glibenclamide, chlorpropamide and tolbutamide in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1985**, *339*, 203–209.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 2 mL water + 200 µL 1 (?) M HCl + 200 µL 2.5 µg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 µL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject a 120 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb ODS 2

Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM
Retention time: 4.2
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: glyburide (glibenclamide), glipizide, tolazamide, tolbutamide

KEY WORDS
serum; derivatization; serum

REFERENCE
Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Decant ether layer and evaporate it to dryness under a stream of nitrogen at 35-40°. Reconstitute extract in 100 μL mobile phase, vortex 30 s, inject 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 6.09
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: glyburide, gliclazide, glipizide, tolbutamide, tolazamide
Noninterfering: trimethoprim, sulfamethoxazole

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 100 μg/mL tolbutamide in water + 500 μL 100 mM HCl + 3 mL dichloromethane, mix for 15 s, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 1000 × 8 10 μm radial pak C18 (Waters)
Mobile phase: MeOH:0.2% acetic acid 60:40 adjusted to pH 6.7 with 1 M NaOH (Wash with MeCN at 1 mL/min for 20 min at the end of each day.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: tolbutamide (11)
Limit of detection: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Bakare,M.T.; Mustapha,A.; Abdu-Aguye,I. An improved high-performance liquid chromatographic determination of chlorpropamide in human plasma, *Chromatographia*, **1994**, *39*, 107–109.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 3.85
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazo-

cine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μL Plasma or 200 μL urine + 100 μL water + 100 μL 1 M HCl + 4 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 2-3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 7 μm BST C8 (BST, Budapest)
Mobile phase: MeCN:isopropanol:0.1% orthophosphoric acid 17:17:66
Flow rate: 1.2
Injection volume: 5
Detector: UV 235

CHROMATOGRAM

Retention time: 8.5
Internal standard: chlorpropamide

OTHER SUBSTANCES

Extracted: tolbutamide

KEY WORDS

plasma; chlorpropamide is IS

REFERENCE

Csillag,K.; Vereczkey,L.; Gachalyi,B. Simple high-performance liquid chromatographic method for the determination of tolbutamide and its metabolites in human plasma and urine using photodiode-array detection, *J.Chromatogr.*, **1989**, *490*, 355–363.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.657

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Grind tablets, weigh out amount equivalent to 45-55 mg chlorpropamide, add 70-80 mL mobile phase, shake for 6-8 min, make up to 100 mL with mobile phase, dilute an aliquot to 0.05 mg/mL with mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5-6 μm Zorbax ODS

Mobile phase: MeCN:1% acetic acid 48:52

Flow rate: 1.5

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: k' 1.8

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

rugged; tablets

REFERENCE

Everett,R.L. Liquid chromatographic determination of chlorpropamide in tablet dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 519–521.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline,

tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.40 (A), 6.34 (B)

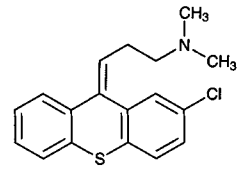
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chlorprothixene



Molecular formula: $C_{18}H_{18}ClNS$
Molecular weight: 315.87
CAS Registry No.: 113-59-7
Merck Index: 2241
Lednicer No.: 1 399

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 5 µg/mL thioridazine + 2 mL water + 2 mL 2 M NaOH, mix well, add 10 mL heptane:isoamyl alcohol 99:1, shake slowly on a reciprocating shaker for 15 min, centrifuge at 5-10° at 1207 g for 5 min. Remove 8.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 µL MeCN:water 60:40, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm LC-PCN (cyano) (Supelco)
Mobile phase: MeCN:20 mM pH 4.5 KH_2PO_4 60:40
Column temperature: 40
Flow rate: 2
Injection volume: 50
Detector: UV 229 or E, IBM Model 230, Model 3892 glassy carbon electrode, 1000 mV vs saturated calomel electrode

CHROMATOGRAM

Retention time: 7.6
Internal standard: thioridazine (8.5)
Limit of quantitation: 5 ng/mL (UV)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; UV and electrochemical detection have about same sensitivity

REFERENCE

Brooks,M.A.; DiDonato,G.; Blumenthal,H.P. Determination of chlorprothixene and its sulfoxide metabolite in plasma by high-performance liquid chromatography with ultraviolet and amperometric detection, *J.Chromatogr.*, **1985**, *337*, 351–362.

SAMPLE

Matrix: blood
Sample preparation: Work under yellow light. 1 mL Serum + 2 mL water + 2 mL 2 M NaOH, vortex for 10 s, add 5 mL water-saturated n-heptane:isoamyl alcohol 99:1, shake gently for 20 min, centrifuge at 4° at 2800 g, remove organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under reduced pressure. Dissolve the residue in 500 µL MeCN, inject a 30 µL aliquot (store at 5°).

HPLC VARIABLES

Column: 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: MeCN:pyridine:140 mM sodium acetate pH 3.1 698:2:300
Flow rate: 0.9
Injection volume: 100

Detector: E, Environmental Sciences Assoc. Coulochem II, Model 5011 detector cell, oxidative screen mode, screen electrode +0.5 V, sample electrode +0.85 V

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: promethazine, methotrimeprazine (levomepromazine)

KEY WORDS
serum; recirculate mobile phase

REFERENCE
Bagli,M.; Rao,M.L.; Höflich,G. Quantification of chlorprothixene, levomepromazine and promethazine in human serum using high-performance liquid chromatography with coulometric electrochemical detection, *J.Chromatogr.B*, **1994**, *657*, 141–148.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Whole blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry under suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL freshly prepared ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Asahipak ODP-50
Mobile phase: MeCN:50 mM ammonium acetate 85:15
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, Finnigan MAT TSP-2 interface, collision gas argon 3.5 mTorr, collision offset -17.5 V, repeller 70 V, vaporizer 130-5°, source 200°, filament off, multiplier 1500 V, dynode power 15 kV, scantime 1.20 s, MSMSC factor 0, monitor 316–271. (The effluent from the column was mixed with 50 mM ammonium acetate pumped at 0.6 mL/min. The mixture flowed to the detector.)

CHROMATOGRAM
Retention time: 4.50
Limit of detection: 0.1 ng

OTHER SUBSTANCES
Extracted: flupenthixol, thiothixene, zuclopenthixol

KEY WORDS
whole blood; SPE

REFERENCE
Verweij,A.M.A.; Hordijk,M.L.; Lipman,P.J.L. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquilizers of the thioxanthene group in whole-blood, *J.Liq.Chromatogr.*, **1994**, *17*, 4009–4110.

SAMPLE
Matrix: bulk
Sample preparation: Prepare solutions in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4 3 μm Hypersil C18-BDS
Mobile phase: MeCN:MeOH:water 40:5:55 containing 6 g/L KH_2PO_4, 2.9 g/L sodium lauryl sulfate, and 9 g/L tetra-*n*-butylammonium bromide
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: 8.66
Limit of detection: 300 ng/mL
Limit of quantitation: 900 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Duignan,G.M.; Miller,J.H.M.B.; Skellern,G.G. Development of a liquid chromatographic method for the control of related substances in chlorprothixene hydrochloride, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 451–456.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine,

metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 202, 225, 264, 324

CHROMATOGRAM

Retention time: 6.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, carbamazepine, chlordiazepoxide, clonazepam, caffeine, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50

Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: triflupromazine, carphenazine, methotrimeprazine, promazine, perphenazine, deserpidine, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 17.60 (A), 8.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, pra-

zosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

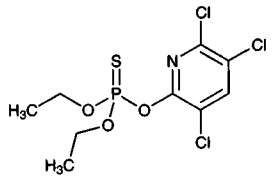
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Chlorpyrifos



Molecular formula: $C_9H_{11}Cl_3NO_3PS$
Molecular weight: 350.59
CAS Registry No.: 2921-88-2
Merck Index: 2242

SAMPLE

Matrix: bile, blood, tissue, urine
Sample preparation: Blood. Extract three time with MeCN:phosphoric acid 99:1, centrifuge. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute, inject an aliquot. Urine. Inject directly. Bile. Dilute 1:4 with water, inject an aliquot. Tissue. Homogenize whole fish, extract three times with 10 mL acetone:phosphoric acid 99:1. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute, inject an aliquot.

HPLC VARIABLES

Column: RCM 100 C18 (Waters)
Mobile phase: Gradient. A was MeCN:water:phosphoric acid 10:89:1. B was MeCN:phosphoric acid 99:1. A:B 0:100 for 3 min, to 25:75 over 12 min, maintain at 25:75 for 10 min.
Flow rate: 1.5
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Retention time: 23

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

fish; catfish; pharmacokinetics

REFERENCE

Barron,M.G.; Plakas,S.M.; Wilga,P.C. Chlorpyrifos pharmacokinetics and metabolism following intravascular and dietary administration in channel catfish, *Toxicol.Appl.Pharmacol.*, **1991**, *108*, 474–482.

SAMPLE

Matrix: blood
Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 µL MeOH:water 70:30, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 µA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 µm, collector slit 400 µm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, negative-ion mode

CHROMATOGRAM
Retention time: 17
Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: chlorpyrifos-methyl, disulfoton, EPN, ethion, fenitrothion, methidathion, parathion, parathion-methyl

KEY WORDS
serum; SPE; m/z 330

REFERENCE
Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize mouse brain in ten volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Plasma or homogenate + 1 g NaCl + 2 mL ethyl acetate + coumaphos, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen.

HPLC VARIABLES
Column: 300 × 4 μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Retention time: 3.3
Internal standard: coumaphos (10.0)
Limit of detection: 40 ng

OTHER SUBSTANCES
Extracted: parathion

KEY WORDS
plasma; rat; mouse; microsomes; brain; normal phase

REFERENCE
Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Determination of organophosphorus insecticides, their oxygen analogs and metabolites by high pressure liquid chromatography, *Chromatographia*, **1982**, *15*, 669–671.

SAMPLE
Matrix: food
Sample preparation: 30 g Rice + 50 mL MeOH, let stand for 48 h with occasional manual shaking, remove a 1 mL aliquot of the MeOH layer and evaporate it to near dryness under a stream of nitrogen, add 1 mL hexane, shake, repeat extraction. Combine the hexane layers and add them to a Sep-Pak Florisil SPE cartridge, elute with 3 mL acetone:hexane 40:60. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak (Waters)
Column: 250 × 3.9 Nova-Pak C18

Mobile phase: Gradient. MeCN:water from 40:60 to 70:30 over 12 min or Isocratic MeCN: water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 18 (gradient), 10 (isocratic) (for chlorpyrifos-methyl)
Limit of detection: 600 ng/g

OTHER SUBSTANCES
Extracted: methacrifos, fenitrothion, etrimfos, carbaryl, pirimphos-methyl (UV 247)

KEY WORDS
rice; SPE

REFERENCE
Brayan,J.G.; Haddad,P.R.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *447*, 249–255.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM
Retention time: 0.61

OTHER SUBSTANCES
Also analyzed: amitraz (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

REFERENCE
Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

SAMPLE
Matrix: soil
Sample preparation: 20 g Air-dried soil + 2 mL water, mix, add 50 mL acetone, shake mechanically at 280 excursions/min for 30 min, filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 5 μm Spherex amino-derivatized silica (Phenomenex)
Mobile phase: Hexane:THF 97:3
Flow rate: 1.5
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Limit of quantitation: 50 ppb

KEY WORDS
comparison with GC/MS and immunoassay

REFERENCE
Hill,A.S.; Skerritt,J.H.; Bushway,R.J.; Pask,W.; Larkin,K.A.; Thomas,M.; Korth,W.; Bowmer,K. Development and application of laboratory and field immunoassays for chlorpyrifos in water and soil matrices, *J.Agric.Food Chem.*, **1994**, *42*, 2051–2058.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize mouse brain with 10 volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Homogenate + 1 g NaCl + 2 mL coumaphos in ethyl acetate, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction with 2 mL ethyl acetate. Combine the ethyl acetate layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Internal standard: coumaphos

KEY WORDS
mouse; brain; normal phase; pharmacokinetics

REFERENCE
Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Factors involved in the differential acute toxicity of the insecticides chlorpyrifos and methyl chlorpyrifos in mice, *Toxicol.Appl.Pharmacol.*, **1982**, *65*, 144–152.

SAMPLE
Matrix: water
Sample preparation: Condition a C8 SPE cartridge with 10 mL MeOH and 10 mL water. Acidify water to pH 2.2 with concentrated HCl, filter (0.45 μm). Add 100 mL water to the SPE cartridge at 5-6 mL/min, dry under vacuum for 30 min, elute with four 500 μL portions of MeOH at 5-6 mL/min, make up the volume of the eluate to 2 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 20 mm long 37-75 μm Porasil B
Column: 250 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeOH:water 82:18
Flow rate: 1
Injection volume: 20-25
Detector: UV 230

CHROMATOGRAM
Retention time: 14.6
Limit of detection: 5 ppb

OTHER SUBSTANCES
Extracted: pendimethalin

KEY WORDS
SPE

REFERENCE
Bogus,E.R.; Watschke,T.L.; Mumma,R.O. Utilization of solid-phase extraction and reversed-phase and ion-pair chromatography in the analysis of seven agrochemicals in water, *J.Agric.Food Chem.*, **1990**, *38*, 142–144.

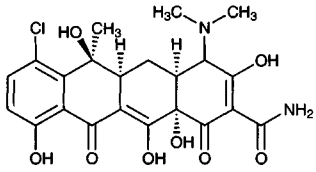
SAMPLE
Matrix: water
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 5 mL MeOH and 10 mL water. Add 250 mL water to the SPE cartridge, elute with 2 mL ethyl acetate, inject a 25 μL aliquot.

HPLC VARIABLES
Column: Ultremex C18
Mobile phase: MeCN:water 80:20
Flow rate: 1.1
Injection volume: 25
Detector: UV 224

KEY WORDS
SPE

REFERENCE
Hill,A.S.; Skerritt,J.H.; Bushway,R.J.; Pask,W.; Larkin,K.A.; Thomas,M.; Korth,W.; Bowmer,K. Development and application of laboratory and field immunoassays for chlorpyrifos in water and soil matrices, *J.Agric.Food Chem.*, **1994**, *42*, 2051–2058.

Chlortetracycline



Molecular formula: $C_{22}H_{23}ClN_2O_8$
Molecular weight: 478.89
CAS Registry No.: 57-62-5, 64-72-2 (HCl)
Merck Index: 2245
Lednicer No.: 1 212

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 200 μL 24% trichloroacetic acid in MeOH + 300 μL mobile phase buffer (A), vortex for 1 min, centrifuge at 2000 g for 15 min, inject 50 μL of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Capcell C18 type SG-120 (Shiseido)
Mobile phase: MeOH:buffer 55:45 (Buffer (A) was 100 mM pH 6.5 sodium acetate containing 35 mM calcium chloride and 25 mM disodium ethylenediamine tetraacetate.)
Column temperature: 30 ± 0.2
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 11
Limit of detection: 35 ng/mL

OTHER SUBSTANCES

Also analyzed: tetracycline, oxytetracycline

KEY WORDS

serum

REFERENCE

Iwaki,K.; Okumura,N.; Yamazaki,M. Rapid determination of tetracycline antibiotics in serum by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1993**, *619*, 319–323.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 1 mL buffer, mix for 30 s, add 6 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. Urine. 100 μL Urine + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 400 μL buffer, mix for 30 s, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. (Buffer was 27.6 g NaH_2PO_4 + 25.2 g sodium sulfite in 100 mL water, pH 6.1.)

HPLC VARIABLES

Column: 100 × 2 5 μm Lichrosorb RP8

Mobile phase: MeCN:100 mM citric acid 24:76
Flow rate: 0.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 7
Internal standard: demeclocycline (4)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: oxytetracycline, tetracycline, methacycline, doxycycline

KEY WORDS
serum

REFERENCE
De Leenheer,A.P.; Nelis,H.J.C.F. Doxycycline determination in human serum and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1979**, *68*, 999–1002.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10 mg/mL solution of tetracycline hydrochloride in water, inject a 20 μL aliquot. Prepare a 10 mg/mL solution of tetracycline in 100 mM HCl, inject a 20 μL aliquot. Formulations. Shake 500 mg capsule blend with 15 mL water and 1 mL concentrated ammonia until solid has dissolved, make up to 50 mL with pH 4.0 phosphate buffer, let stand for 10 min, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: Gradient. A was MeCN:water:phosphoric acid 240:1650:27, adjust pH to 2.2 with 45% KOH, make up to 2000 with water. B was MeCN:water:phosphoric acid 440:1500:27, adjust pH to 2.2 with 25% KOH, make up to 2000 with water. A:B 100:0 for 10 min then 0:100 for 5 min.
Flow rate: 2.6
Injection volume: 20
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Simultaneous: impurities, tetracycline

REFERENCE
Muhammad,N.; Bodnar,J.A. Separation and quantitation of chlortetracycline, 4-epitetracycline, 4-epianhydrotetracycline, and anhydrotetracycline in tetracycline by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 928–930.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. Ointment. Dissolve 250 mg ointment in 30 mL diethyl ether, extract with three 25 mL portions of 10 mM HCl, combine the extracts and make up to 100 mL with 10 mM HCl, inject an aliquot. (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Laboratories)
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

capsules; tablets; ointment

REFERENCE

Bryan,P.D.; Stewart,J.T. Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 675–692.

SAMPLE

Matrix: eggs, tissue
Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH: water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES

Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)
Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM

Retention time: 19

Limit of detection: 0.92 ng/g (pig kidney), 0.66 ng/g (pig muscle), 1.05 ng/g (cow liver), 0.39 ng/g (chicken egg)
Limit of quantitation: 4 ng/g (pig kidney)

OTHER SUBSTANCES
Extracted: doxycycline, oxytetracycline, tetracycline
Also analyzed: demeclocycline

KEY WORDS
cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE; post-column reaction

REFERENCE
Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S
Column: 150 × 4.6 5 μm Polymer Labs PLRP-S
Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).
Flow rate: 1
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 27.3
Limit of detection: 6 ng/g

OTHER SUBSTANCES
Extracted: tetracycline, oxytetracycline, demeclocycline

KEY WORDS
chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE

Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE

Matrix: feed

Sample preparation: Weigh out 10 or 20 g feed, add 100 mL acid-acetone solution, shake for 2 min. Adjust the pH to less than 1.2 with HCl, shake, repeat until the pH is stable, shake for 45 min. Centrifuge at 2500 rpm for 5 min and filter. Dilute the filtrate with acid-acetone solution, inject a 20 μL aliquot. (Acid-acetone solution was acetone:water:4 M HCl 65:30:5.)

HPLC VARIABLES

Guard column: C18 Alltima (Alltech)

Column: 150 × 4.6 5 μm C18 Alltima (Alltech)

Mobile phase: Gradient. A was MeOH. B was buffer. A:B 35:65 for 5 min, to 60:40 over 8 min, maintain at 60:40 for 7 min, re-equilibrate at initial conditions for 5 min (Buffer was 100 mM sodium acetate containing 50 mM calcium chloride and 25 mM disodium EDTA, adjusted to pH 6.5 with concentrated HCl or 40% NaOH.)

Flow rate: 1.5

Injection volume: 20

Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 12.5

Limit of quantitation: 2.5 μg/g

OTHER SUBSTANCES

Noninterfering: amprolium, arsanilic acid, decoquinate, monensin, oxytetracycline, roxarsone, sulfamethazine, sulfathiazole, penicillin

KEY WORDS

comparison with UV 365

REFERENCE

Houglum,J.E.; Larson,R.D.; Knutson,A. Assay of chlortetracycline in animal feeds by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 961–965.

SAMPLE

Matrix: feed

Sample preparation: 20 g Feed + 100 mL 4 M HCl:acetone:water 1:8:6, shake mechanically for 45 min, allow to settle for 10 min, centrifuge an aliquot at 3500 rpm for 10 min, filter the supernatant (A) (Millex-HV) (reject first 8-10 drops), add 3 mL filtrate to an unconditioned J.T. Baker C18 SPE cartridge, slowly force through, reject the first few drops, inject a 20 μL aliquot of the eluate. (If necessary wash 10 mL supernatant (A) + 3.6 mL water saturated with dichloromethane with 3.6 mL dichloromethane saturated with water, filter (Millex-HV) the aqueous layer, inject an aliquot.)

HPLC VARIABLES

Column: 300 × 3.9 Nova-Pak C18

Mobile phase: MeCN:MeOH:10 mM oxalic acid 1.5:1:3.5, adjust pH to 2.0 with 7.2 M HCl

Flow rate: 1

Injection volume: 20

Detector: UV 370

CHROMATOGRAM

Retention time: 5

KEY WORDS
SPE

REFERENCE
Holland,D.C.; Faul,K.C.; Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of chlortetracycline hydrochloride in ruminant and poultry/swine feeds, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 780–784.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve ointment in petroleum ether, add an equal volume of EtOH:water 70:30, dilute with MeOH to 100 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 5:95 containing 1.3 mM disodium citrate, 1 mM tetrabutylammonium bromide, 1.1 mM citric acid, and 8 mM EDTA.
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.43

OTHER SUBSTANCES
Simultaneous: anhydrotetracycline, demeclocycline, doxycycline, epianhydrotetracycline, oxytetracycline, quatrimycin, rolitetracycline, tetracycline

KEY WORDS
ointment

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: honey
Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: demeclocycline (demethylchlortetracycline), doxycycline, methacycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: honey
Sample preparation: Prepare a 100 mg Baker 10 C18 cartridge by washing with MeOH, water, and 10 mL saturated aqueous Na_2EDTA. Dissolve 5 g honey in 20 mL 100 mM pH 4.0 Na_2EDTA-McIlvaine buffer, filter, apply to cartridge, wash with 20 mL water, air dry under vacuum for 5 min. Condition a Baker 10 COOH cartridge with ethyl acetate. Elute contents of C18 cartridge onto COOH cartridge with 50 mL ethyl acetate. Wash COOH cartridge with 10 mL MeOH, elute with 10 mL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Bakerbond C8
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 1:1.5:3
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Simultaneous: tetracycline, doxycycline, oxytetracycline

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Uchiyama,M.; Asukabe,H.; Mori,Y.; Suzuki,M. Improvement of chemical analysis of antibiotics. IX. A simple method for residual tetracyclines analysis in honey using a tandem cartridge clean-up system, *J.Chromatogr.*, **1987**, *389*, 417–426.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 7.9

OTHER SUBSTANCES
Extracted: demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; cow; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: milk
Sample preparation: 2 mL Milk + 4 mL buffer, filter (Amicon CF-25 ultrafiltration membrane) while centrifuging at 20° at 1000 g for 1 h, suspend solids in 2 mL buffer and repeat filtration for 40 min. Combine filtrates and inject a 500 μL aliquot as soon as possible. (Buffer (McIlvaine) was prepared by mixing 625 mL 28.41 g/L Na_2HPO_4 and 1 L 21.01 g/L citric acid monohydrate. The buffer was also 100 mM in disodium EDTA and the final pH was 4.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 10 min, re-equilibrate at 0:0:100 at 1.5 mL/min for 5 min and at 1 mL/min for 1 min. (Flush daily with 10 column volumes of water. Store column in MeOH:water 60:40, flush with water before use.)
Column temperature: 30
Flow rate: 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 13.1
Limit of detection: 19.8 ng/mL
Limit of quantitation: 51.9 ng/mL

OTHER SUBSTANCES
Extracted: oxytetracycline, tetracycline

KEY WORDS
cow; protect from light; ultrafiltrate

REFERENCE
Thomas,M.H. Simultaneous determination of oxytetracycline, tetracycline, and chlortetracycline in milk by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 564–567.

SAMPLE

Matrix: milk

Sample preparation: Place 22 g 40 μm, 18% load, end-capped bulk C18 material (Analytichem) in a 50 mL syringe barrel, wash with 2 column volumes hexane, dichloromethane, and MeOH, vacuum aspirate until dry. 2 g Bulk C18 material + 50 mg disodium EDTA + 50 mg oxalic acid + 500 μL milk + 10 μL MeOH, blend gently in a glass mortar and pestle for 30 s, place the mixture in a 10 mL plastic syringe barrel plugged with a piece of filter paper. Compress column volume to 4.5 mL, add a 100 μL pipette tip on the column outlet to restrict the flow. Wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL MeCN:ethyl acetate 75:25. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 300 × 4 10 μm Micro Pak ODS
Mobile phase: MeCN:10 mM oxalic acid in water 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: 5.8
Limit of detection: 2 ng

OTHER SUBSTANCES

Extracted: tetracycline, oxytetracycline

KEY WORDS

cow; matrix solid-phase dispersion

REFERENCE

Long,A.R.; Hsieh,L.C.; Malbrough,M.S.; Short,C.R.; Barker,S.A. Matrix solid-phase dispersion (MSPD) isolation and liquid chromatographic determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 379–384.

SAMPLE

Matrix: milk

Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.

Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM
Retention time: 15.3
Limit of detection: 1.27 ng/mL
Limit of quantitation: 2.35 ng/mL

OTHER SUBSTANCES
Extracted: demeclocycline, doxycycline, methacycline, minocycline, oxytetracycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS
cow; SPE; ultrafiltrate

REFERENCE
Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 1 mL 1 M HCl, mix, add 24 mL MeCN slowly with swirling over 30 s, let stand for 5 min, decant the clear supernatant through a plug of glass wool. 15 mL Filtrate + 15 mL dichloromethane + 30 mL hexane, mix, collect the aqueous layer. Extract the organic layer with 1 mL water. Combine the aqueous layers, make up to 4 mL with water, filter (13 mm, 0.45 μm, PVDF), inject a 1000 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Column: 150 × 4.6 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Mobile phase: Gradient. MeCN:buffer 20:80 for 3 min, to 38:62 over 22 min, maintain at 38:62 for 5 min, return to initial conditions for 1 min, re-equilibrate for 9 min. (Buffer was 3.94 g potassium oxalate + 3.61 g oxalic acid + 1.22 g sodium decanesulfonate in 1 L water, pH 2.30.)
Flow rate: 1
Injection volume: 1000
Detector: UV 365

CHROMATOGRAM
Retention time: 23
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: tetracycline, oxytetracycline

REFERENCE
White,C.R.; Moats,W.A.; Kotula,K.L. Optimization of a liquid chromatographic method for determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.AOAC Int.*, **1993**, *76*, 549–554.

SAMPLE
Matrix: milk
Sample preparation: Prepare a column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass

column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Centrifuge 10 mL milk at 2100 g for 5 min, decant the skimmed milk, rinse the tube with two 1 mL portions of water. Add 10 mL pH 4.0 buffer to the milk and rinses, sonicate for 3 min, filter (Whatman 541 paper) the supernatant. Add the filtrate to the column, wash with 2 mL pH 4.0 buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, add 700 μL EDTA buffer to the column, elute with 3 mL EDTA buffer, add 20 μL 25 μg/mL demeclocycline hydrochloride in MeOH to the eluate, inject a 100 μL aliquot. (Prepare pH 4.0 buffer by adjusting 100 mM succinic acid to pH 4.0 with 10 M NaOH. Prepare EDTA buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 29.2 g NaCl, and 100 mmoles EDTA in 1 L water.)

HPLC VARIABLES

Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 5 μm 100 Å PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:buffer 15:10:60 (Buffer was 10 mM oxalic acid adjusted to pH 2.0 with 4 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water.)

CHROMATOGRAM

Retention time: 11.7
Internal standard: demeclocycline (8.3)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: oxytetracycline, tetracycline

KEY WORDS

protect from light; cow; post-column reaction; derivatization; SPE; complexation

REFERENCE

Croubels,S.; Van Peteghem,C.; Baeyens,W. Sensitive spectrofluorimetric determination of tetracycline residues in bovine milk, *Analyst*, **1994**, *119*, 2713–2716.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM

Retention time: 6.3

OTHER SUBSTANCES

Simultaneous: furazolidone, oxytetracycline, tetracycline

REFERENCE

Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 10 mM HCl, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 5 μm PLRP-S styrene-divinyl benzene copolymer (Polymer Laboratories)
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 355

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μg/mL solution in 10 mM HCl.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 5 μm PLRP-S styrene-divinylbenzene copolymer (Polymer Laboratories)
Mobile phase: Gradient. MeCN:50 mM pH 2.0 oxalate buffer 15:85, for 3 min to 60:40 over 17 min, maintain at 60:40 for 5 min, return to initial conditions over 1 min, re-equilibrate for 9 min. (After use flush with water for 10 min, store in MeCN:water 60:40.)
Flow rate: 1
Detector: UV 355

CHROMATOGRAM
Retention time: 15.5

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline

REFERENCE
White,C.R.; Moats,W.A.; Kotula,K.L. Comparative study of high performance liquid chromatographic methods for the determination of tetracycline antibiotics, *J.Liq.Chromatogr.*, **1993**, *16*, 2873–2890.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g

wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0. Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with 1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES

Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S
Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM

Retention time: 25
Limit of detection: 20 μg/kg

OTHER SUBSTANCES

Extracted: demeclocycline, oxytetracycline, tetracycline

KEY WORDS

SPE; sheep; cattle; liver; kidney; column-switching

REFERENCE

Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 479

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Extracted: doxycycline, oxytetracycline, tetracycline

KEY WORDS
cow; SPE; kidney; liver; muscle

REFERENCE
Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-.; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

SAMPLE
Matrix: tissue
Sample preparation: Prepare an affinity column by filling a 10 mL column with 5 mL chelating Sepharose, allow to settle, wash with 20 mL 0.5% copper(II) sulfate solution, eliminate air bubbles by agitation, wash with 15 mL 50 mM pH 4 succinate buffer, do not allow to dry. Condition an Analytichem Bond Elut C18 SPE cartridge with 10 mL MeOH and 10 mL water, do not allow to dry. Homogenize 4 g minced kidney with 40 mL 50 mM pH 4 succinate buffer, sonicate for 10 min, centrifuge at 9000 rpm for 10 min, filter the supernatant through paper, repeat the extraction. Combine the supernatants and pass them through the affinity column at 5-7 mL/min, wash with 10 mL water, wash with 30 mL MeOH, wash with 20 mL water, elute with 50 mL 50 mM pH 4 succinate buffer containing 3.7% Titriplex III (ethylenedinitrilotetracacetic acid, disodium salt dihydrate). Add the eluate to the SPE cartridge at 5-7 mL/min, wash with 10 mL water, dry with air aspiration for 10 min, elute with 5 mL MeOH:MeCN 1:1, evaporate the eluate at 40° under a stream of nitrogen, dissolve the residue in 500 µL mobile phase, inject an aliquot. Protect from light through process. (The affinity columns may be re-used up to 15 times by washing with 20 mL water then 20 mL EtOH:water 20:80 then conditioning as described above.)

HPLC VARIABLES
Guard column: Perisorb RP-8
Column: two 300 × 100 5 µm Chromspher C8 columns (cat. no. 28262) in series
Mobile phase: MeCN:10 mM pH 2 oxalic acid 20:80
Flow rate: 0.8
Detector: UV 365

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 30 ng/g

OTHER SUBSTANCES
Simultaneous: tetracycline, oxytetracycline, demethylchlortetracycline, methacycline, doxycycline

KEY WORDS
kidney; SPE

REFERENCE
Degroodt,J.M.; Wyhowski de Bukanski,B.; Srebrnik,S. Multiresidue analysis of tetracyclines in kidney by HPLC and photodiode array detection, *J.Liq.Chromatogr.*, **1993**, *16*, 3515–3529.

SAMPLE
Matrix: tissue

Sample preparation: Condition a 500 mg Separcol SI C18 SPE cartridge (Anapron) with 2 mL MeOH and 4 mL buffer. Homogenize 5 g muscle with 20 mL buffer and 3 mL n-hexane:dichloromethane 1:3 at 4°, centrifuge at 2400 g at 4° for 30 min, remove the supernatant, repeat homogenization with 10 mL buffer. Combine the supernatants, slowly add with constant stirring a volume of 1 g/mL trichloroacetic acid in water equal to 10% of the supernatant volume, stir for another min, keep in ice for 15 min, filter through paper, add to SPE cartridge at no more than 10 mL/min, wash with 2 mL water, elute with 4 mL 10 mM oxalic acid in MeOH, inject a 10 µL aliquot. (Buffer was 15 g $Na_2HPO_4.2H_2O$ + 13 g citric acid monohydrate + 3.72 g EDTA in 1 L water, pH 4.)

HPLC VARIABLES

Guard column: 5 µm LiChrospher 100 RP-18 guard column
Column: 250 × 4 5 µm HP Spherisorb ODS 2
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 20:35:45
Flow rate: 1
Injection volume: 10
Detector: UV 360

CHROMATOGRAM

Retention time: 8.5
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Simultaneous: tetracycline, oxytetracycline

KEY WORDS

muscle; cow; pig; SPE

REFERENCE

Sokol,J.; Matisova,E. Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction, *J.Chromatogr.A*, **1994**, *669*, 75–80.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut cyclohexyl (CH) SPE cartridge with 10 mL MeOH and 10 mL water. Powder (domestic food blender) frozen kidney or muscle. Homogenize (Silverson Machines) 5 g powdered tissue and 45 mL 100 mM glycine in 1 M HCl for 1 min, add 5 g ammonium sulfate, shake for 30 s, let stand for 10 min, centrifuge at 2000 rpm for 15 min, filter (glass wool) the supernatant, repeat the extraction with 50 mL 100 mM glycine in 1 M HCl. Combine the filtrates and centrifuge an aliquot at 2200 rpm for 10 min, add a 20 mL aliquot of the supernatant to the SPE cartridge, wash with 10 mL water, elute with 7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 65°, reconstitute the residue in 500 µL MeCN:20 mM oxalic acid 20:80, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: Chromspher C8 (Chrompack)
Column: 200 × 3 5 µm Chromspher C8 glass column (Chrompack)
Mobile phase: Gradient. A was MeCN. B was MeCN:20 mM oxalic acid 10:90. A:B from 10:90 to 20:80 over 2 min, maintain at 20:80 for 8 min, to 25:75 over 1 min, maintain at 25:75 for 9 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 0.4
Injection volume: 50
Detector: F ex 390 em 490 following post-column reaction. The column effluent mixed with 750 mM aluminum chloride (degas by sonication, store in a brown bottle) pumped at 0.6 mL/min and flowed through a 13.7 m × 0.3 mm i.d. PTFE column at 60° to the detector.

CHROMATOGRAM

Retention time: 20.4
Limit of detection: 70 ng/g (kidney), 30 ng/g (muscle)

OTHER SUBSTANCES

Extracted: oxytetracycline, tetracycline

KEY WORDS

pig; cow; poultry; kidney; muscle; SPE; post-column reaction; complexation

REFERENCE

McCracken,R.J.; Blanchflower,W.J.; Haggan,S.A.; Kennedy,D.G. Simultaneous determination of oxytetracycline, tetracycline and chlortetracycline in animal tissues using liquid chromatography, post-column derivatization with aluminium, and fluorescence detection, *Analyst*, **1995**, *120*, 1763–1766.